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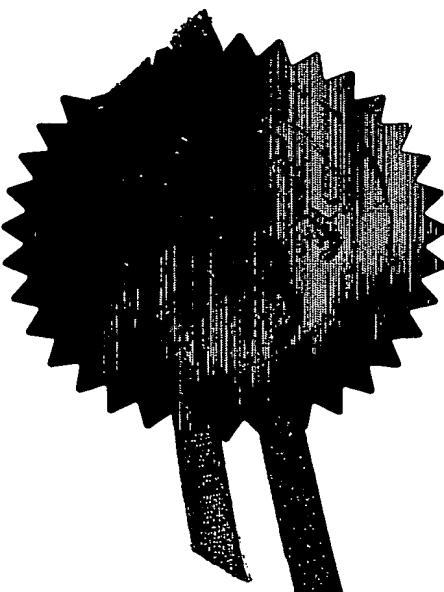
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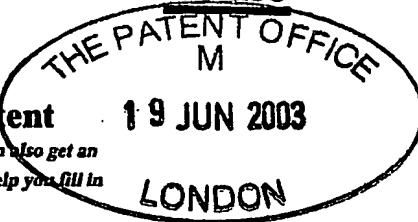
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Cardiff Road
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A1528

2. Patent application number

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0314341.9

19 JUN 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

ABERDEEN UNIVERSITY
RESEARCH AND INNOVATION
UNIVERSITY OFFICE
KINGS COLLEGE
ABERDEEN AB24 3FX
UK

Patents ADP number (if you know it)

8642183001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

A METHOD FOR PRODUCING A DI-SUBSTITUTED PYRIDINIUM COMPOUND

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent
(including the postcode)

ABLETT & STEBBING
CAPARO HOUSE
101-103 BAKER STREET
LONDON
W1U 6FQ

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Country	Priority application number (if you know it)	Date of filing (day / month / year)
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A METHOD FOR PRODUCING A DI-SUBSTITUTED PYRIDINIUM COMPOUND

The present invention relates to a method for producing a di-substituted pyridinium compound.

5

Dialkylpyridinium compounds, (Di-APS), in particular 1,3-APS oligomers, are known to be produced by sponges, for example the Haploscerid genera such as *Haliclona*, *Amphimedon* and *Callyspongia*, as part of their chemical defences, and have 10 potentially useful biological properties. Diverse biological activities have been identified for different 1,3-APS compositions, including cytotoxicity, neurotoxicity and inhibition of action potentials, stimulation of transmitter release, inhibition of K⁺ conductances, and anticholinesterase 15 activity. At least some of these observed actions of 1,3-APS compositions relate to the pore forming or membrane lesion effects of these compounds, which properties may be useful, for example, in the transfection of cells with genetic material.

20

Naturally occurring 1,3-APS compounds are produced as cocktails of different 1,3-APS compounds, from which individual 1,3-APS compounds are difficult to isolate, due to the same basic structure and very similar molecular weights 25 of the different 1,3-APS compounds. However, it is desirable to be able to isolate different individual 1,3-APS compounds, in order to determine their different biological activities, for example with regard to the effect of degree of polymerisation, and length and rigidity of linking chains.

30

An alternative approach to isolating individual naturally occurring Di-APS compounds, is to synthesise these compounds by laboratory methods. Di-APS compounds generally occur as

high molecular weight linear oligomers, having a molecular weight ranging from 1 KDa to greater than 25 KDa, having varying lengths of aliphatic chains linking the pyridine units. However, model linear Di-APS compounds closely 5 analogous to the natural products remain to be synthesized by laboratory methods in a controllable fashion.

Previous studies have succeeded in producing linear 1,3-APS oligomers, but the methods employed meant that the product 10 obtained consisted of a mixture of linear and cyclic oligomers with a wide range of molecular weights (see for example, Davies-Coleman, M.T.; Faulkner, D.J. *J. Org. Chem.* 1993, 58, 5925-5930). The approach used in these studies was to synthesize the 3-substituted pyridine monomer and introduce 15 a good leaving group, for example bromide, at the end of the alkyl chain. This monomer was then refluxed to initiate polymerisation. Alternative methods based on this strategy incorporate an ether linkage in the linking chain (Gil, L. et al *Tetrahedron Lett.* 1995, 36, 2059-2062), or form cyclic 20 dimers (Morimoto, Y.; Yokoe, C. *Tetrahedron Lett.* 1997, 38, 8981-8984) or tetramers with short linking chains (Shinoda, S. et al, *Chem. Commun.* 1998, 181).

A further study for producing macrocyclic 1,3-APS compounds 25 is disclosed in Kaiser, A. et al., *J. Am. Chem. Soc.* 1998, 120, 8026-8034, which involves the formation and subsequent reaction of an N-(2,4-dinitrobenzene) pyridinium salt (Zinke salt) to yield linear 1,3-APS compounds. However, this method has certain disadvantages, in that it is relatively complex, 30 and has only been shown to work for small macrocyclic oligomers.

Accordingly, there still exists a need for a reliable method

for synthesizing linear di-substituted pyridinium compounds, for example di-APS oligomers and polymers. The present invention seeks to provide such a method, which overcomes the aforementioned disadvantages of previous methods.

5

According to the present invention there is provided a method of producing a linear di-substituted pyridinium compound, the method comprising the steps of:

(a) attaching a first 2, 3, or 4-substituted pyridine 10 compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{X}$ (1) to a solid support, to form a compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{Y-SUPPORT}$ (2), wherein SUPPORT represents the solid support, R is selected from hydrogen, hydroxyl, and substituted or unsubstituted alkyl, alkoxy, aryl, alkaryl, aralkyl, and alkenyl groups, R' is a 15 first linking group, X is a group which can react with the solid support to attach the first pyridine compound to the support, and Y is absent or is a second linking group;

(b) forming a di-substituted pyridine compound of the formula $-\text{A}-\text{NC}_5\text{R}_4-\text{R}'-\text{Z}$ (3) from a second 2, 3, or 4-substituted 20 pyridine compound of formula (1), which second pyridine compound may be the same as or different to the first pyridine compound, wherein A is a protecting group, and Z is a leaving group;

(c) reacting the compound of formula (2) formed in step 25 (a) with the compound of formula (3) formed in step (b), to form a di-substituted pyridinium compound of the formula $-\text{A}-\text{NC}_5\text{R}_4-\text{R}'-[-\text{Q}^+\text{NC}_5\text{R}_4-\text{R}'-]_n\text{Y-SUPPORT}$ (4), wherein Q⁺ is a counter ion and n=1;

(d) optionally, repeating step (c) as many times as 30 required to obtain a compound of formula (4) wherein n is an integer of 2 or greater; and

(e) detaching the compound of formula (4) from the solid support, and removing the protecting group A to form a

di-substituted pyridinium compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{[Q}^+\text{NC}_5\text{R}_4-\text{R}'\text{-}]_n\text{-X}$ (5), wherein n is an integer, and Q^+ and X are a counter ion and a group which can react with the solid support to attach the first pyridine compound to the support respectively, which may be the same or different as Q and X defined above in steps (c) and (a) respectively.

Thus, during steps (a) to (d) of the method of the present invention, one end of the compound which is to become the product compound of formula (5) is bound to a solid support, thereby removing it from the reaction equilibrium, and substantially eliminating cyclisation. The use of protecting and leaving groups per the method of the present invention allows for the controlled addition of monomer units without macrocycle formation occurring in solution. The method of the present invention can be used to produce di-substituted pyridinium compounds in high rates of conversion, and in high yields. The method of the present invention also has the advantage that a variety of linking groups may be used, which may incorporate different functionalities, for example functionalities which can restrict the conformational space accessible by the di-substituted pyridinium compound, such as a double bond or cyclopropyl ring, or groups which allow the attachment of fluorescent labels, so that the di-substituted pyridinium compound can be localised intracellularly and in cell membranes.

Herein the term "di-substituted pyridinium compound" can refer to either a 1,2-, 1,3-, or 1,4-substituted pyridinium compound. However, the preferred products of the method of the present invention are 1,3-substituted pyridinium compounds, in particular linked 1,3-dialkyl pyridinium compounds.

Although the method of the present invention is suitable for producing oligomers and polymers potentially having a wide range of degrees of polymerisation, and hence molecular weights, for example n=1 to 20 in formula (5) above, it is particularly useful for producing oligomers having higher degrees of polymerisation, for example n=20 to 100 in formula (5) above.

Referring to the steps of the method of the present invention 10 in turn, in step (a) a first 2,3,or 4-substituted, preferably 3-substituted, pyridine compound of the formula $\text{NC}_5\text{R}_4\text{-R}'\text{-X}$ (1) is attached to a solid support, to form a compound of the formula $\text{NC}_5\text{R}_4\text{-R}'\text{-Y-SUPPORT}$ (2), wherein SUPPORT represents the solid support, R is selected from hydrogen, and substituted 15 or unsubstituted alkyl, aryl, alkaryl, aralkyl, and alkenyl groups, R' is a first linking group, X is a group which can react with the solid support to attach the first pyridine compound to the support, and Y is absent or is a second linking group. Each R group is preferably a hydrogen atom.

20

Compounds of formula (1) are commercially available, or may be synthesised as is known in the art. Thus, for example, a compound of formula (1) may be synthesised by reaction of a compound of formula $\text{Z}'\text{-R}''\text{-X}$ with a suitable pyridine compound, 25 with protection of the X- group as necessary, wherein R'' is a linking group and Z' is a suitable leaving group. For example, a compound of formula (1) may be prepared by reacting $\text{Br-R}''\text{-OH}$ (i.e. $\text{Z}'=\text{Br}$ and $\text{X}=\text{OH}$) with t-butyldimethyl-chlorosilane (TBDMSCl) to form $\text{Br-R}''\text{-OTBDMS}$, which may be 30 reacted with 2,3,or 4-methylpyridine (2,3, or 4-picoline) with deprotection of the X group to form $\text{NC}_5\text{H}_4\text{-R-OH}$, wherein R' is as defined above, i.e. a linking group equivalent to R'' plus one carbon atom. Of course, protecting groups other than

TBDMSCl may be used, as will be apparent to those skilled in the art.

As referred to above, the method of the present invention is 5 advantageous in that a variety of linking groups may be used, which may incorporate different functionalities. Preferred R' groups have no terminal carbon atoms, which further restricts the possibility of cyclisation occurring.

10 Thus, in formulas (1) to (5) above, each group R' may be the same or different and selected from an alkylene group (for example, a group $-(\text{CH}_2)_m-$, wherein m is an integer from 2 to 12, preferably from 6 to 10), an alkenyl-containing group (for example, a group having from 2 to 12 carbon atoms, preferably 15 from 6 to 10 carbon atoms, containing one or more alkenyl groups, e.g. cis- or trans- 2-butenyl, 3-hexenyl, 2,5-hepten-di-yl, and 4-octenyl groups), a cyclopropanyl-containing group (for example, cis- or trans- $-(\text{CH}_2)_p-\text{cyclopropyl}-(\text{CH}_2)_q-$ wherein p and q are the same or different and are integers 20 from 1 to 4, preferably 1 or 2). As discussed above, R' may also be a group which comprises a fluorescent label, so that the di-substituted pyridinium compound can be localised intracellularly and in cell membranes (for example, a linking group R' having a pendant alcohol group, for attachment of a 25 fluorescent group).

Where appropriate, cis- or trans- isomerism may be introduced into linking group R' as is known in the art (for example, Morimoto, Y.; Yokoe, C. *Tetrahedron Lett.* 1997, 38, 8981-30 8984). A double bond may be converted into a cyclopropyl ring using carbene chemistry.

In the compound of formula (1), X is a group which can react

with the solid support to attach the compound of formula (1) to the support. Thus, the particular group X to be used in a particular compound of formula (1) will depend upon the particular solid support being used. Thus, suitable X groups 5 include hydroxyl, carboxyl, thiol, and amine groups. However, X is preferably a hydroxyl group, and particularly preferred compounds of formula (1) are pyridines 3-substituted by a hydroxyalkyl group, i.e. compounds of the formula $\text{NC}_5\text{R}_4-(\text{CH}_2)_n-\text{OH}$, wherein n is as defined above.

10

The solid support used in the present invention may be any suitable support to which the compound of formula (1) may be attached. Preferred solid support materials are organic resins having functionality which can react with group X of the 15 compound of formula (1), described above. Particularly preferred solid support materials are trityl chloride and functionalised polystyrene resins (for example, Merrifield resins, i.e. chloromethylstyrene-divinylbenzene resins).

20 Group Y in formula (2) is absent or a second linking group, depending on the particular reaction which occurs between the compound of formula (1) and the solid support to form the compound of formula (2). Thus, group X in formula (1) may be eliminated, or, for example, in the case of a compound of 25 formula (1) in which X is a hydroxyl group, the compound of formula (2) may have the more specific formula $\text{NC}_5\text{R}_4-\text{R}'-\text{O-SUPPORT}$, i.e. Y may be an oxygen atom.

In step (b) of the method of the present invention, a 30 disubstituted pyridine compound of the formula $\text{A}-\text{NC}_5\text{R}_4-\text{R}'-\text{Z}$ (3) is formed from a second 2,3 or 4-substituted pyridine compound of formula (1), which second pyridine compound may be the same as or different to the first pyridine compound,

wherein A is a protecting group, and Z is a leaving group. Step (b) is independent of step (a), and accordingly may be performed before, after, or simultaneously with step (a).

5 Step (b) itself thus includes two steps: a first step of converting group X to a leaving group Z, for reaction with the nitrogen atom of the pyridine group of the compound of formula (2), and a second step of protecting the nitrogen atom of the second pyridine compound, with a protecting group A.

10

In step (b), group X may be converted to a suitable leaving group Z as is known in the art. For example, as a hydroxyl group, X may be converted to a mesyl (methanesulphonyl) group by reaction with mesyl chloride. Other suitable leaving groups 15 will be apparent to those skilled in the art.

The nitrogen atom of the second pyridine compound of formula (1) used in step (b) may be protected by conversion to the N-oxide as is known in the art, i.e. protecting group A is 20 oxygen, for example by reaction of the nitrogen atom of the pyridine group with a peracid, for example m-chloroperbenzoic acid. Alternatively, the nitrogen atom may be protected by formation of the borane, i.e. A is BH_3^- .

25 In step (c) of the method of the present invention, the compound of formula (2) formed in step (a) is reacted with the compound of formula (3) formed in step (b), to form a di-substituted pyridinium compound of the formula $-\text{A}-\text{NC}_5\text{R}_4-\text{R}'-[\text{-Q}^+\text{NC}_5\text{R}_4-\text{R}'-]_n\text{Y-SUPPORT}$ (4), wherein Q⁺ is a counter 30 ion and n=1. Thus, in step (c) two pyridine-containing monomer units are combined to form a dimeric di-substituted pyridinium compound, the leaving group Z of the compound of formula (3) reacting with the nitrogen atom of the pyridine group of the

compound of formula (2). The reaction may take place on heating, in the presence of a suitable counter ion Q^- , for example a chloride, iodide, or other suitable counter ion.

5 In step (d) of the method of the present invention, step (c) is optionally repeated as many times as required to obtain a compound of formula (4) wherein n is an integer of 2 or greater. If the desired di-substituted pyridinium compound end product is a dimer (i.e. a compound of formula (5) in which 10 n=1), then no repetitions of step (c) are required. However, as discussed above, the method of the present invention is particularly useful for producing oligomers having higher degrees of polymerisation, for example n=20 to 100 in formula (5) above, which will of course require the repetition of step 15 (c) the appropriate number of times.

In preferred embodiments of the present invention, chain extension to produce oligomers having a higher degree of polymerisation may be accelerated by releasing oligomers 20 having a lower degree of polymerisation from the solid support as compounds having the formula (5), and reintroducing them as reagents as an alternative to, or in addition to, the second pyridine compound used in step (b). Thus, in these preferred embodiments, a compound of formula 25 $NC_5R_4-R'-[Q^+NC_5R_4-R'-]_n-X$ (5) may be converted to a compound of formula $A^--[Q^+NC_5R_4-R'-]_n-Z$ (5a) per step (b), and the compound of formula (5a) may then be reacted with the compound of formula (2) formed in step (a) or (c), per step (d).

30 In step (e) of the method of the present invention, the compound of formula (4) is detached from the solid support, and reduced to form a di-substituted pyridinium compound of the formula $NC_5R_4-R'-[Q^+NC_5R_4-R'-]_n-X$ (5), wherein n is an

integer, and Q⁻ and X are a counter ion and a group which can react with the solid support to attach the first pyridine compound to the support respectively. Q⁻ and X of step (e) may be the same or different as Q⁻ and X defined above with reference steps (a) and (c) respectively. The compound of formula (4) may be detached from the solid support according to the particular solid support being used. Thus, in the case of a functionalised trityl chloride resin solid support, the compound of formula (4) may be detached using a strong acid.

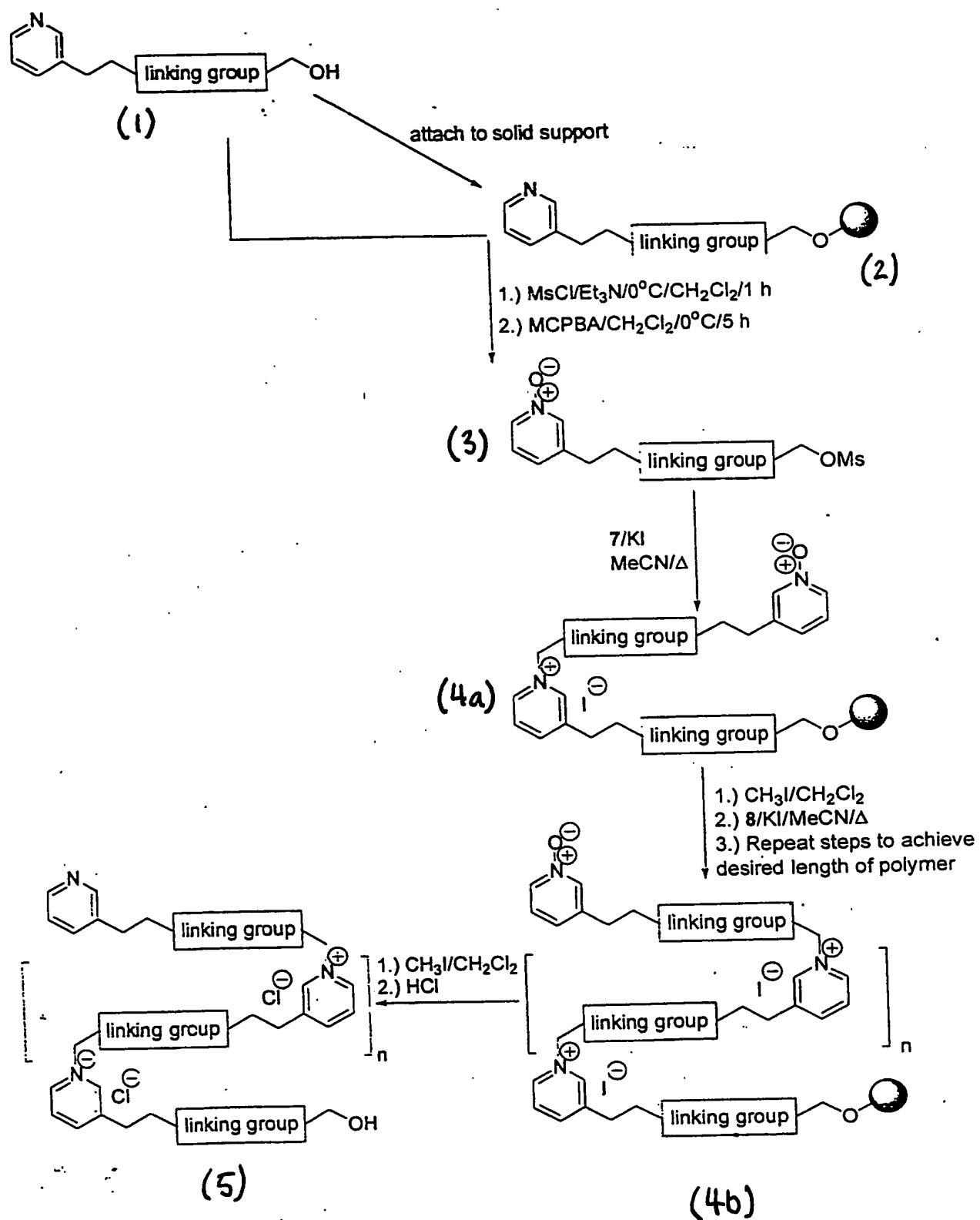
10 For example, if the strong acid used to detach the formula (4) from the solid support is hydrochloric acid, then counter ion Q⁻ will be chloride. The protecting group A can be removed as is known in the art, for example by reducing the N-oxide to a nitrogen atom where A in formula (4) is oxygen.

15

An embodiment of the present invention will now be described in detail.

Reaction Scheme 1 below describes a preferred method for
20 producing a preferred linear 1,3-alkylpyridinium compound of the present invention.

Scheme 1



wherein: Ms=methanesulphonyl (mesyl)
Et=ethyl
MCPBA = m-chloroperbenzoic acid

5 Referring to Scheme 1, 3-substituted pyridine compound (1) is formed by firstly reacting Br-R"-OH with t-butyldimethylchlorosilane (TBDMSCl) to form Br-R"-OTBDMS, wherein R" is a suitable linking group (described hereinabove). The Br-R"-OTBDMS is then reacted with 3-methylpyridine 10 (3-picoline) with deprotection of the X group to form NC₅R₄-R'-OH (3-substituted pyridine compound (1)), wherein R' is a linking group equivalent to R" plus one carbon atom.

A first molecule of 3-substituted pyridine compound (1) is 15 then attached to a trityl chloride resin solid support, to form compound (2).

Before, after, or simultaneously with the formation of compound (2), a second molecule of 3-substituted pyridine 20 compound (1) is reacted firstly with mesyl chloride, and secondly with m-chloroperbenzoic acid to form compound (3) of formula -O-⁺NC₅R₄-R'-O-Ms, wherein Ms is a mesyl group.

Next, compound (2) is reacted with one equivalent of compound 25 (3) to form 1,3-alkylpyridinium compound (4a) having the formula -O-⁺NC₅R₄-R'-I⁺NC₅R₄-R'-O-SUPPORT, i.e. two pyridine-containing monomer units are combined to form a dimeric 1,3-alkylpyridinium compound, the -Ms leaving group of compound (3) reacting with the nitrogen atom of the pyridine group of 30 compound (2). This step is repeated the appropriate number of times to obtain an 1,3-alkylpyridinium compound end product having the desired degree of polymerisation, i.e. compound (2) is reacted with 1,3-alkylpyridinium compound (4a) to produce

compound (4b) of formula $\text{O}^- \text{NC}_5\text{R}_4-\text{R}' - [\text{I}^+ \text{NC}_5\text{R}_4-\text{R}' -]_n \text{O-SUPPORT}$, wherein n is preferably 20 to 100.

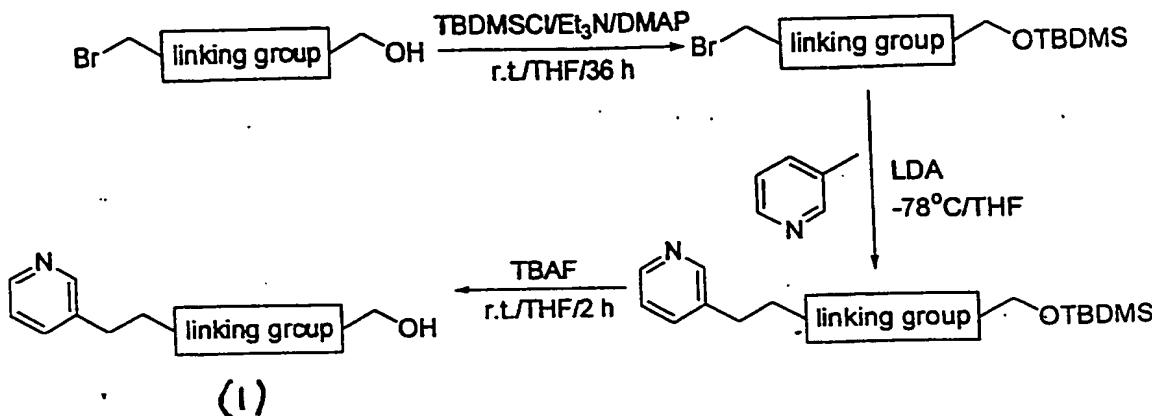
As discussed above, in preferred embodiments of the present invention, chain extension to produce oligomers having a higher degree of polymerisation may be accelerated by releasing oligomers having a lower degree of polymerisation from the solid support as compounds having the formula (5), and reintroducing them as reagents as an alternative to, or 10 in addition to, the second pyridine compound used in step (b). Thus, in these preferred embodiments, a compound of formula $\text{NC}_5\text{R}_4-\text{R}' - [\text{Q}^+ \text{NC}_5\text{R}_4-\text{R}' -]_n - \text{X}$ (5) may be converted to a compound of formula $\text{O}^- \text{NC}_5\text{R}_4-\text{R}' - [\text{Q}^+ \text{NC}_5\text{R}_4-\text{R}' -]_n - \text{Z}$ (5a) per step (b), and the compound of formula (5a) may then be reacted with the compound 15 of formula (2) formed in step (a) or (c), per step (d).

Finally in Scheme 1, compound (4b) is detached from the solid support, and reduced to form 1,3-alkylpyridinium compound (5) of formula $\text{NC}_5\text{R}_4-\text{R}' - [\text{Cl}^+ \text{NC}_5\text{R}_4-\text{R}' -]_n - \text{OH}$ using hydrochloric acid, 20 as shown. The counter ion in final product (5) is thus chloride.

As referred to above, the method of the present invention is advantageous in that a variety of linker groups may be used, 25 which may incorporate different functionalities.

Reaction Scheme 2 below describes a method for producing a compound from which starting material $\text{NC}_5\text{R}_4-\text{R}' - \text{X}$ used in step (a) of the present invention may in turn be produced, in which 30 R' is an alkenyl-containing linking group having either cis- or trans- isomerism.

Scheme 2



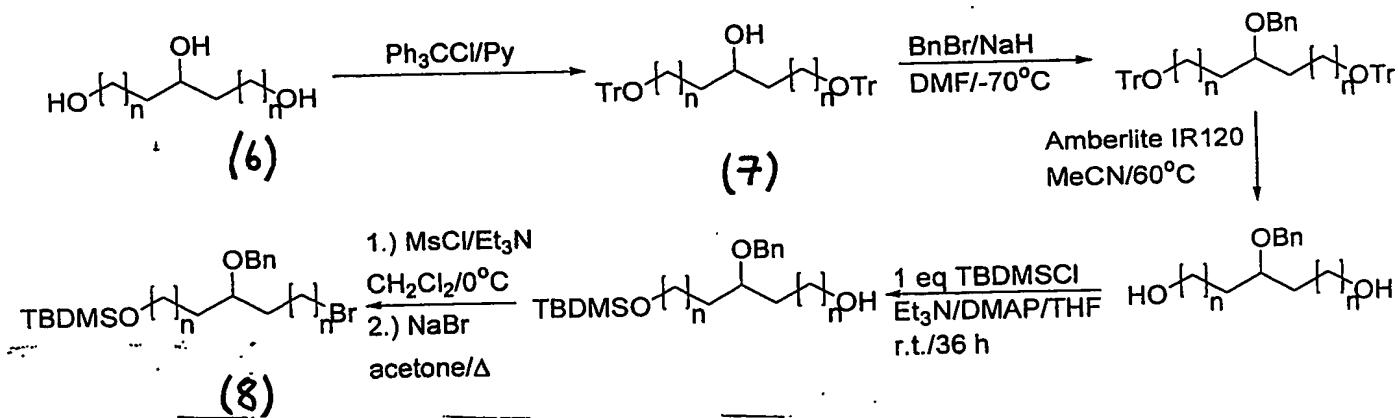
wherein:
TBDMS=t-butyldimethylsilyl
DMAP=4-dimethylaminopyridine
THF=tetrahydrofuran
LDA=lithium diisopropylamide
TBAF=t-butylammonium fluoride

15

Reaction Scheme 3 below describes a method for producing a compound from which starting material NC₅R₄-R'-X of formula
20 (1) used in step (a) of the present invention may in turn be
produced, in which R' is an alkylene linking group having a
pendant alcohol group to which a fluorescent group may be
attached.

25

Scheme 3



wherein: Ph=phenyl
Py=pyridine
Tr=trityl
Bn=benzyl

5

As shown in Scheme 3, triol (6) is differentially protected to form alcohol (7), which is subsequently converted to bromide (8), which can then in turn be used to form starting material NC₅R₄-R'-X of formula (1) used in step (a) of the 10 present invention.

Fluorescent labels, available commercially, can be reacted with the monomer or oligomer, depending on the reaction conditions necessary. To avoid cell damage, the excitation 15 wavelength of the fluorescent label is preferably above 350 nm, and the emission wavelength should preferably be greater than 80 nm higher, to permit ratiometric measurements. Suitable fluorescent labels include fluorescein, lissamine, and rhodamine based compounds, although other fluorescent 20 materials may be used according to conditions. Such a linking group having a fluorescent group would preferably be incorporated into the 1,3-APS compound end product only once or twice.

CLAIMS

1. A method of producing a linear di-substituted pyridinium compound, the method comprising the steps of:

5 (a) attaching a first 2, 3, or 4-substituted pyridine compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{X}$ (1) to a solid support, to form a compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{Y-SUPPORT}$ (2), wherein SUPPORT represents the solid support, R is selected from hydrogen, hydroxyl, and substituted or unsubstituted alkyl, 10 alkoxy, aryl, alkaryl, aralkyl, and alkenyl groups, R' is a first linking group, X is a group which can react with the solid support to attach the first pyridine compound to the support, and Y is absent or is a second linking group;

15 (b) forming a di-substituted pyridine compound of the formula $-\text{A}-\text{NC}_5\text{R}_4-\text{R}'-\text{Z}$ (3) from a second 2, 3, or 4-substituted pyridine compound of formula (1), which second pyridine compound may be the same as or different to the first pyridine compound, wherein A is a protecting group, and Z is a leaving group;

20 (c) reacting the compound of formula (2) formed in step (a) with the compound of formula (3) formed in step (b), to form a di-substituted pyridinium compound of the formula $-\text{A}-\text{NC}_5\text{R}_4-\text{R}'-[-\text{Q}^+\text{NC}_5\text{R}_4-\text{R}'-]_n\text{Y-SUPPORT}$ (4), wherein Q⁺ is a counter ion and n=1;

25 (d) optionally, repeating step (c) as many times as required to obtain a compound of formula (4) wherein n is an integer of 2 or greater; and

30 (e) detaching the compound of formula (4) from the solid support, and reducing to form a di-substituted pyridinium compound of the formula $\text{NC}_5\text{R}_4-\text{R}'-[-\text{Q}^+\text{NC}_5\text{R}_4-\text{R}'-]_n-\text{X}$ (5), wherein n is an integer, and Q⁺ and X are a counter ion and a group which can react with the solid support to attach the first pyridine compound to the support respectively, which may be the

same or different as Q and X defined above in steps (c) and (a) respectively.

2. A method according to claim 1 wherein each R group is 5 hydrogen.

3. A method according to claim 1 or 2 wherein in step (d), step (c) is repeated such that in formula (5) n=20 to 100.

10 4. A method according to claim 1, 2 or 3 wherein the compound of formula (1) is prepared by reaction of a compound of formula Z'-R"-X with a pyridine compound, with protection of the X- group as necessary, wherein R" is a linker group and Z' is a suitable leaving group.

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5. A method according to claim 4 wherein the compound of formula (1) is prepared by reacting Br-R"-OH with t-butyldimethyl-chlorosilane (TBDMSCl) to form Br-R"-OTBDMS, which is reacted with 2,3 or 4-methylpyridine (2,3, or 4-picoline) with deprotection of the X group to form NC₅R₄-R'-OH.

6. A method according to any one of claims 1 to 5 wherein linker group R' has no terminal carbon atoms.

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7. A method according to any preceding claim wherein each group R' is the same or different and is selected from an alkylene group, an alkenyl-containing group, and a cyclopropanyl-containing group.

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8. A method according to claim 7 wherein each group R' is the same or different and is selected from a group -(CH₂)_m-, wherein m is an integer from 2 to 12, a group having from 2

to 12 carbon atoms containing one or more alkenyl groups, an
cis- or trans- -(CH₂)_p-cyclopropanyl-(CH₂)_q- wherein p and q
are the same or different and are integers from 1 to 4.

5 9. A method according to any preceding claim wherein R' is
a group which comprises a fluorescent group, or a group to
which a fluorescent group can be attached.

10. A method according to claim 9 wherein R' has a pendant
10 alcohol group, for attachment of a fluorescent group.

11. A method according to any preceding claim wherein in
formula (1) X is selected from hydroxyl, carboxyl, thiol, and
amine groups.

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12. A method according to claim 11 wherein X is a hydroxyl
group.

13. A method according to claim 12 wherein the compound of
20 formula (1) is a compound of the formula NC₅R₄-(CH₂)_n-OH.

14. A method according to any preceding claim wherein the
solid support material comprises an organic resin having
functionality which can react with group X of the compound of
25 formula (1).

15. A method according to claim 14 wherein the solid support
material comprises trityl chloride or a functionalised
polystyrene resin.

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16. A method according to any preceding claim wherein group
Y in formula (2) is an oxygen atom.

17. A method according to any preceding claim wherein in step
(b) group X is converted to a mesyl (methanesulphonyl) group
by reaction with mesyl chloride.

5 18. A method according to any preceding claim wherein A is
oxygen or BH₃-.

19. A method according to claim 18 wherein A is oxygen, and
the nitrogen atom of the second pyridine compound of formula
10 (1) used in step (b) is converted to the N-oxide by reaction
of the nitrogen atom of the pyridine group with a peracid.

20. A method according to claim 19 wherein the peracid
comprises m-chloroperbenzoic acid.

15

21. A method according to any preceding claim wherein counter
ion Q⁻ in step (c) is an iodide ion.

22. A method according to any preceding claim wherein
20 oligomers having the formula (5) are released from the solid
support and reintroduced as reagents as an alternative to, or
in addition to, the second pyridine compound used in step (b).

23. A method according to claim 22 wherein a compound of
25 formula NC₅R₄-R'-[Q⁺NC₅R₄-R'-]_n-X (5) is converted to a
compound of formula A-Q⁺NC₅R₄-R'-[Q⁺NC₅R₄-R'-]_n-Z (5a) per step
(b), and the compound of formula (5a) is then reacted with the
compound of formula (2) formed in step (a) or (c), per step
(d).

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24. A method according to any preceding claim wherein the
compound of formula (4) is detached from the solid support,
and reduced to form a di-substituted pyridinium compound of

the formula $\text{NC}_5\text{R}_4-\text{R}'-\text{[Q}^+\text{NC}_5\text{R}_4-\text{R}'-\text{]}_n-\text{X}$ (5) using an acid.

25. A method according to claim 24 wherein the acid is hydrochloric acid, and counter ion Q^- is chloride.

5

26. A method according to any preceding claim wherein the di-substituted di-substituted pyridinium compound is a linked dialkyl pyridinium compound.

10 26. A method of producing a linear di-substituted pyridinium compound substantially as hereinbefore described with reference to Reaction Schedule 1.

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